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(54) Title: VIRAL SUPPRESSION, TREATMENT AND PREVENTION OF VIRAL INFECTIONS

(57) Abstract

It has been discovered that it is possible to administer neutralizing antibodies produced by a first mammal into a second mammal for the purpose of treating or preventing viral infections, with the first and second mammals being of either the same or different species. The neutralizing antibodies are mixed with the virus of interest such that the neutralizing antibodies bind at least one but not all epitopes of the virus so as to render the virus noninfectious while maintaining immunogenicity. The neutralizing antibodies may be administered as a serum transfusion, a vaccine, or a topical preparation.

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VIRAL SUPPRESSION, TREATMENT AND PREVENTION OF VIRAL INFECTIONS

TECHNICAL FIELD

The present invention relates to passive immunity in relation to viral infection.

BACKGROUND OF THE INVENTION

An immunocompetent animal will produce an immunological response to a foreign protein. The immunological response to the foreign protein predictably comprises both humoral and cellular immunity.

The humoral response produces antibodies which include but are not limited to IgM, IgG and IgA. The antibodies produced may be either of two classes: (a) a neutralizing antibody, i.e., an antibody not requiring the complement system for cellular or viral destruction; and (b) a complement fixing antibody.

The principle of passive immunity provides that immunoglobulin from a different species may be used to prevent infection of another species not of the same type. While an immunoglobulin produced by one member of a species may be used in another member of the same species, the immunoglobulin may also be used in a member of a different species via serum transfusion or administration of a vaccine.

The practice of utilizing vaccinations as a technique for acquiring immunity has occurred since at least the 1700's when Jenner first recognized the correlation between cowpox and the lessening of the virological virulence of smallpox in the milkmaids' population. In this historical event, Jenner inoculated a live cowpox virus into an immunologically healthy boy, and upon subsequent inoculation of the

child with live smallpox virus, the child did not develop life-threatening smallpox. In this instance, the outer membrane of the cowpox virus was so similar to the smallpox virus that the body's immune system could not tell the two apart; thus, antibodies raised against cowpox virus administered to a human also could react against invading smallpox virus. This method required administration of a virus to humans, said virus being disease-causing to bovines but not humans.

It has now been found that the neutralizing antibodies developed in one mammal upon exposure to a virus can be administered to another mammal to provide treatment for the suppression or prevention of viral infections.

SUMMARY OF THE INVENTION

In one aspect of the present invention, a method for producing neutralizing antibodies for the treatment of a viral infection in a human patient is disclosed.

In another aspect, a method for treating a viral infection in a human patient is disclosed.

In another aspect, a virus neutralizing agent for the treatment of a viral infection in a human patient is disclosed.

In yet another aspect, a method for neutralizing a virus expected to contact an area on a human by applying a virus neutralizing agent is disclosed.

In another aspect, a method for neutralizing a virus expected to contact at least one surface of a condom by applying a virus neutralizing agent is disclosed.

In another aspect, a method for neutralizing a virus expected to contact at least one surface of a latex rubber glove by applying a virus neutralizing agent is disclosed.

In another aspect, a method for neutralizing caprine encephalitis virus in livestock is disclosed.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1a is a graph depicting the inhibition of SIV infection expressed as the number of fusion sites in CEMx174 cells by various dilutions of goat anti-SIV serum over time.

Fig. 1b is a graph depicting the SIV infection rate of CEMx174 cells expressed as the number of infusion sites upon exposure to various dilutions of normal goat serum over time. This represents the control data for Fig. 1a.

Fig. 2a is a graph depicting the inhibition of SIV infection expressed as the number of fusion sites in CEMx174 cells by various dilutions of goat anti-SIV serum at Day 2 post-infection.

Fig. 2b is a graph depicting the inhibition of SIV infection expressed as the number of fusion sites in CEMx174 cells by various dilutions of goat anti-SIV serum at Day 3 post-infection.

Fig. 2c is a graph depicting the inhibition of SIV infection expressed as the number of fusion sites in CEMx174 cells by various dilutions of goat anti-SIV serum at Day 5 post-infection.

Fig. 3 is a graph depicting the inhibition of SIV infection expressed as the number of fusion sites in CEMx174 cells by a 1:20 dilution of goat anti-SIV serum over time.

DETAILED DESCRIPTION

Upon creating humoral and cellular immunity in a mammal, introduction of the resulting IgA, IgG1, and/or other neutralizing antibodies and other immunocompetent antibodies and cellular activity on or into a system containing a virus, such as human immunodeficiency virus (HIV), prevents virus replication and renders that virus noninfectious. In so doing, further viral replication may be retarded, blocked, or stopped by means of the IgA, IgG1, other neutralizing antibodies and cellular immune action.

An immunocompetent animal makes antibodies in response to simian immunodeficiency virus (SIV), HIV and/or other viruses. These antibodies react with the virus in an antigen-antibody reaction. The normal response for antibody production is for IgM to be produced first, followed by IgG. The antibodies IgM and IgG are not capable of activating or causing biodestruction of some viruses, such as the SIV or HIV virus. It is believed that the basis for this incompetent antigen-antibody response is that the SIV/HIV virus either produces intrinsically, through either viral synthesis or host cell synthesis, an SIV, HIV or viral complement inhibiting factor which prevents activation of the complement system, i.e., viral destruction. This incompetent antigen-antibody response is itself incapable of activating the body's complement system, because the antibodies IgM and IgG

are known to be complement fixing antibodies. Thus, where the incompetent antigen-antibody response is detected by standard techniques, the incompetent antigen-antibody response can be eliminated or bypassed as an inhibiting factor by the use of antibodies that are of the neutralizing antibody class.

Using this methodology of inserting into mammals viruses for which IgM and IgG may not activate or cause biodestruction such as SIV, HIV, polioviruses, influenza, hanta virus, pox viruses, caprine encephalitis (CAE) virus, herpes-viruses, hepatitis, encephalitis, measles, mumps, Ebola, and/or rubella, serum containing neutralizing antibodies and cellular immunity may be obtained naturally. These products when suitably treated and prepared may yield as a minimum the following: (a) a vaccine suitable for use in humans; (b) an immunological barrier to virus transmission via mucosal surfaces by way of creams, sprays, liquids and swabs; (c) a serum to arrest further viral development; (d) a preparation to be applied directly to mucosal membranes for adherence of the neutralizing antibodies to the mucosa.

Each of these applications for human usage is based upon, but not limited to, the use of a mammal which, upon being exposed to SIV, HIV, or similar viruses, does not die but rather produces an immunological reaction. The mammal's immunological reaction to the introduction of the SIV and/or HIV

virus yields antibodies which are complement fixing or neutralizing antibodies and also cellular immune reactions which when treated in accord with accepted procedures yield the production of a useable serum immunoglobulin which can then be used to prevent further virus replication.

It is known that, in procedures described herein, SIV, HIV, and their immunoglobulin, in particular, IgA, do not affect nonhuman hosts. Roitt, Ivan, *Essential Immunology*, Blackwell Scientific Publications, Cambridge, MA (1991). A preferred nonhuman host used to demonstrate and develop serum or milk containing neutralizing antibodies and cellular immunity is an advanced pregnant female goat. This animal is chosen because it does not die but rather produces large quantities of neutralizing antibodies which can be isolated from serum and/or milk. However, neither a pregnant female goat not a female goat is required. A pregnant female goat is preferred in order to provide milk production as an additional source for IgA, IgG1, and other neutralizing antibodies. Other nonhuman mammals may also be used to obtain the neutralizing antibodies of interest provided that, when exposed to simian SIV, HIV, or similar viruses, they do not die but rather produce humoral and cellular reactions. Although not required, the nonhuman mammal's immunological reaction can also be potentiated by use of an appropriate adjuvant.

Humans who have been exposed to SIV, HIV, and/or similar viruses, and who exhibit humoral and cellular reactions to the virus(es) may also be used as sources of neutralizing antibodies. These neutralizing antibodies may be administered to children and adults either via serum transfusion or as a vaccine prepared from serum or milk. For persons with immature digestive systems which are capable of absorbing antibodies or antibody-making cells, such as newborn infants, the neutralizing antibodies may also be ingested via milk or a modified milk product.

Example I: Preparation of Concentrated Simian Immunodeficiency Virus (SIV)

A macrophage-tropic strain of SIV (SIVmac239-17E) referred to as "SIV-17E" was prepared by growing the virus in CEMx174 cells. Sharma, et al., "Derivation of neurotropic lymphocytotropic parental virus: pathogenesis of infection in macaques," *J Virol* 66: 352-3556 (1992). The CEMx174 cell is an immortalized CD4-bearing human T/B hybrid cell line that is highly susceptible to SIV-induced cytopathicity (fusion) and permissive for replication by SIVmac. Hoxie, et al., "Biological characterization of a simian immunodeficiency virus-like retrovirus (HTLV-IV): Evidence for CD4-associated molecules required for infection," *J Virol* 62:2557-2568 (1988). Koenig, et al., "Selective infection of human CD4+ cells by simian

immunodeficiency virus: productive infection associated with envelope glycoprotein induced fusion," *Proc Natl Acad Sci USA* 86:2443-2447 (1989). The cells were grown in RPMI-1640 medium ("RPMI") supplemented with 10% fetal bovine serum, glutamine and gentamicin, and were used for preparation of stock virus and the virus neutralization assay. Cell cultures (9 milliliter) were inoculated with 1 milliliter of virus (10^4 $TCID_{50}$/milliliter) and examined for cell fusion. When approximately 50% of the cells had fused, the cultures were expanded by the addition of fresh cells. Cultures were further monitored for infectivity by fusion and reverse transcriptase. Supernatant fluids (approximately 240 milliliters) were collected and clarified by centrifugation. The stock contained 10^4 $TCID_{50}$/milliliter in CEMx174 cells. Virus was pelleted at 27,000 rpm is a SW28 rotor (Beckman Instruments, Inc., Fullerton, CA) for two hours at 4°C, resuspended in two milliliters NET buffer (50 mM HCl, 5 mM ethylenediaminetetraacetic acid, 10 mM Tris hydrochloride, pH 7.4) and purified on a Sepharose CL-4B column (Pharmacia Diagnostics, Inc., Fairfield, NJ).

Example II: Neutralization of the SIV Virus In Vitro

Neutralizing antibody or antibodies obtained from a nonhuman mammal exposed to SIV virus were examined for antiviral activity in vitro.

Simian immunodeficiency virus (SIV) was prepared according to procedures given in Example I. The virus was heat-killed in a 60°C water bath for thirty minutes. Killed virus was used for the sake of safety; however, use of live virus will result in a faster immunogenic response.

A pregnant female goat was exposed to the simian virus by intramuscular injection. The goat was injected with a one milliliter suspension of killed SIV at 1 X 10^6 viral particles per milliliter once per week for three weeks. The goat's immunogenic response was augmented using the MPL® (RIBI IMMUNOCHEM RESEARCH, INC.) + TDM Adjuvant System (Sigma Chemical Co., St. Louis, MO) according to the manufacturer's instructions, i.e., administering intramuscularly 500 μl into each hind leg.

Following exposure of the goat to simian SIV virus, the animal's serum and/or milk was obtained. Milk was obtained from the goat as soon as it became available, generally at about three weeks after the birth of the kid. The milk was frozen until subsequent use.

To collect serum, blood samples of at least 10 cc were drawn from a large bore vein after the first week and weekly thereafter for twelve weeks. This regimen was selected to optimize the opportunity to first detect neutralizing antibodies. Each serum sample was obtained by centrifugation of the blood sample, frozen

for transport to the laboratory, and subsequently tested for neutralizing antibodies. The neutralizing antibodies were first detected on or about the eighth week. During subsequent weeks, samples were tested to monitor changes in neutralizing antibody concentration, and the amount of antibodies detected increased over time. Straight, untreated serum (without concentrating the antibodies) obtained from the blood sample taken at the twelfth week was used in the in vitro neutralization studies.

A neutralization assay was performed to demonstrate the ability of the neutralizing antibodies and cellular immunity in the goat serum to prevent infectivity of the SIV virus in vitro. SIV-17E virus (100 $TCID_{50}$/milliliter) was incubated with doubling dilutions of the goat serum at 37°C for one hour. In a 96-well tissue culture plate, 100 microliters of each SIV-17E/goat serum mixture was added to wells using three wells per dilution. Approximately 5 x 10^4 CEMx174 cells were then added to each well. The cultures were incubated at 37°C and observed for fusion over a period of five days. Fusion was observed in control cultures within one day. The neutralizing antibody titer was taken as the highest dilution of serum which prevented cell fusion.

The results of the neutralization assay are given in Table 1 and illustrated in Fig. 1a and 1b as the average number of fusion sites observed over time for

various dilutions of goat anti-SIV serum (Gα-SIV), compared to normal goat serum (NGS) as the control. Fig. 2a, 2b, and 2c present this neutralization data for Day 2, Day 3 and Day 5 post-infection. Fig. 3 depicts neutralization of SIV infection over time by goat anti-SIV serum used at a 1/20 dilution. Table 2 presents the neutralization assay data as percent inhibition of SIV fusion sites by various dilutions of goat anti-SIV serum at Day 2 post-infection.

A 1/20 dilution of the goat anti-SIV serum almost completely inhibited SIV infection of the CEMx174 cells (97.2% inhibition). Dilutions of 1/40 and 1/80 inhibited 92.4% and 83.7%, respectively. These results indicate that the goat anti-SIV serum contains potent neutralizing antibodies which can be used to block the infectivity of the SIV virus.

Table 1
Neutralization of SIV by Goat Anti-SIV Sera
(Reduction of Fusion Sites in CEMX174 Cells)

	Average Number of Fusion Sites							
	Day							
Serum Dilution	1		2		3		5	
	NGS	Gα-SIV	NGS	Gα-SIV	NGS	Gα-SIV	NGS	Gα-SIV
1/20	3.5	0	70	2	80	4.5	175	3.5
1/40	5	1.5	72.5	5.5	90	11.5	187.5	22.5
1/80	5	4.5	75	13	90	18.0	212.5	24.5
1/160	5.5	5.5	80	34	92.5	56.0	225	65
1/320	5.5	5.5	85	58	95.2	68.0	235	80
1/640	6.5	8	85	85	99	92.5	250	100

NGS = Normal goat serum; Gα-SIV = Goat anti-SIV serum

Table 2

Percent Inhibition of SIV Fusion Sites by

Different Dilutions of Goat Anti-SIV Serum

(Day 2 post-infection)

	Dilution	% Inhibition
1	1/20	97.2
2	1/40	92.4
3	1/80	83.7
4	1/160	57.5
5	1/320	31.7
6	1/640	0

A vaccine may be produced according to the procedures outlined in Example II by first exposing the same or a different mammalian species to a virus such as SIV or HIV. The preferred nonhuman mammal is a goat. The resultant neutralizing antibody or antibodies are extracted from the animal's serum or milk via standard methods such as ammonium sulfate or sodium sulfate precipitation and centrifugation methodology followed by purification by such methods as dialysis or gel filtration. Tijssen, P., "Practice and theory of enzyme immunoassays," *Laboratory Techniques in Biochemistry and Molecular Biology*, R.H. Burdon and P.H. van Knippenberg (eds.), Amsterdam: Elsevier Science Publishers, vol.15, pp. 96-98 (1985)

Once the neutralizing antibodies are isolated, placing the neutralizing antibody or antibodies in the presence of live SIV and/or HIV viruses (Brooks, et al., *Medical Microbiology*, 19th ed., Appleton & Lange, East Norwalk, CN, p.150 (1991)) or other viruses into a host species, the SIV and/or viral cells are rendered noninfectious and incapable of further replication. This process of using neutralizing antibody or antibodies to attenuate the SIV, HIV or other virus is referred to as an antibody attenuation of a virus to produce a vaccine, or "AAV2".

Example III: Preparation and Use of a Vaccine

Neutralizing antibody or antibodies are used to attenuate the SIV, HIV, and other viruses to produce a vaccine.

The HIV virus is extracted from a human donor who has been diagnosed as being HIV+ and whose HIV virus is both isolated and sero-typed. (Bobkov, et al., "Identification of an *env* G subtype and heterogeneity of HIV-1 strains in the Russian Federation and Belarus," *AIDS* 8:1649-1655(1994); Gao, et al., "Genetic variation of HIV type 1 in four World Health Organization-sponsored vaccine evaluation sites: generation of functional envelope (glycoprotein 160) clones representative of sequence subtypes A, B, C, and E," *AIDS Res Hum Retroviruses* 10:1359-1368 (1994); Kaleebu, et al., "Identification of HIV-1 subtype G

from Uganda," *AIDS Res Hum Retroviruses* 11:657-659 (1995); WHO Network for HIV Isolation and Characterization, "HIV type 1 variation in World Health Organization-sponsored vaccine evaluation sites: genetic screening, sequence analysis, and preliminary biological characterization of selected viral strains," *AIDS Res Hum Retroviruses* 10:1327-1343 (1994); Delwart, et al., "Genetic relationships determined by a DNA heteroduplex mobility assay: analysis of HIV-1 *env* genes," *Science* 262:1257-1261 (1993)) The HIV virus of the human donor is then placed on or injected into a nonhuman mammal such as a goat. This placement or injection into the goat produces an immunological response in the form of neutralizing antibodies. The neutralizing antibodies are extracted from the mammal's serum or milk and then mixed with the human donor's HIV sero-typed virus to produce the attenuated antibody viral vaccine, or AAV2.

The proportion of virus to antibody requires that antibody is bound to at least one epitope on each virus particle but that not all epitopes are bound. It is believed that attachment of a single neutralizing antibody to a single epitope is sufficient to prevent fusion, and thus, virulence is removed while immunogenicity is maintained. For a given virus-neutralizing antibody(ies) combination, the neutralization assay described in Example II can be performed to determine what ratio of neutralizing

antibody to virus results in complete neutralization of the virus. A vaccine is then prepared using a neutralizing antibody concentration of less than that which resulted in complete neutralization as determined in the neutralization assay.

The AAV2 is in the form of an HIV-IgA complex which maintains immunogenicity but neutralizes virulence. The AAV2 is then returned, typically by injection, back into the human donor. Placement of the AAV2 into the human donor produces a competent immunological response which blocks, prevents, or destroys further progress or development of the SIV and/or HIV virus into the AIDS complex disease.

It is noted that IgA and other antibodies are mixed with the human donor virus in a effective amount so as to allow immunogenicity to be attained and maintained by bonding some but not all viral epitopes, thereby yielding an immunological response different from the parent virus but still achieving neutralization of the SIV/HIV virus by rendering it an attenuated noninfectious virus (AAV2). The binding of all epitopes will destroy immunogenicity and virulence. This process yields a vaccine, or AAV2, which, when placed into a human body having an identical sero-typed HIV virus, denies further viral replication thereby allowing the human body's immune system to be stimulated to increase its own production of competent antibodies as a result of AAV2 stimulation. This increase of the human body's competent antibodies then

prevents the additional production or replication of the simian SIV or human HIV virus and the development or continuation of the AIDS complex disease.

Modern vaccination technologies are also well known in the art. For example, recombinant DNA vaccines have been produced for viruses such as cholera, herpes simplex, HIV, and hepatitis B. These vaccines provide selected immunogenic peptides which can also be coupled with adjuvants. Immunogenic viruslike particles (VLP) comprising proteins of the same size and structure as viruses but without the viral DNA or RNA can be produced by fusing foreign genes to the Ty gene in yeast. Methodologies for producing vaccines of chemically synthesized peptides which comprise the primary structure of antigenic regions of an infectious virus are also available. Also known in the art are idiotypic vaccines prepared by exposing a host to a classical antigen, allowing the host to produce antibodies (Ab-1) to the antigen, then allowing the host to produce antibodies (Ab-2) to the original antibodies (Ab-1), with the antibodies (Ab-2) having antigenic determinants resembling the original antigen and demonstrating improved immunogenicity by maintaining the tertiary structure of the antigen. (Coleman, et al., *Fundamental Immunology*, 2d ed., Wm C. Brown Publishers, 1992; Keeton, W.T. and Gould, J.L., *Biological Science*, W.W. Norton, 1993; Solomon, et al.,

Biology, 3d ed., Saunders College Publishing, Harcourt Brace Jovanovich College Publishers, 1983) It is foreseen that recombinant DNA vaccines, VLP's, synthetic peptides, or idiotypic vaccines, rather than live virus, can be used in the present invention to produce neutralizing antibodies in a host mammal.

Synthetic peptides derived from the complementarity-determining region (CDR) sequences of antibodies can be mass-produced in vitro which are similar to the intact antibody, inhibitory to idiotype-anti-idiotype interactions, bind specific antigens, interact with cellular receptors, and stimulate biological processes. (Taub, et al., *J. Biol Chem* 264:259 (1989); Bruck, et al., *Proc Natl Acad Sci USA* 83:6578 (1986); Kang, et al., *Science* 240:1034 (1988); Williams, et al., *Proc Natl Acad Sci USA* 86:5537 (1989); Novotny, et al., *J Mol Biol* 189:715 (1986)) Although these peptide analogs seem to have a limited use in vivo due to their proteinaceous characteristics such as water insolubility, high immunogenicity, their ability to adopt various conformations, and subjectability to proteolysis, it is foreseen that such synthetic peptides might be used in the present invention.

It is well known in the art that a monoclonal antibody can be mass-produced via hybridoma technology for the purpose of economically providing large amounts of a vaccine. Virus neutralizing agents in the form of

monoclonal antibodies can also be utilized in the present invention. In this technique, neutralizing antibody producing cells are fused with immortal cells of a myeloma to produce the hybridoma cells; the hybridoma cells are screened for antibody production; the cells which produce the desired monoclonal antibody are then either cultured in large numbers in tissue culture or reinjected into the peritoneal cavities of many mice where the cells multiply and produce large quantities of monoclonal antibody in the ascites fluid that is formed; the ascites fluid containing the monoclonal antibody is collected; and the monoclonal antibody is purified by techniques such as affinity chromatography or column chromatography.

Techniques for producing non-peptide compounds referred to as mimetics have been developed which permit the synthesis of a conformationally restricted molecule that mimics the binding and functional properties of monoclonal antibodies. The mimetics are synthesized by first determining the relevant contact residues and conformation involved in the antibody-antigen binding (Williams, et al., *J Biol Chem* 266:5182 (1991); Segal, et al., *Proc Natl Acad Sci USA* 71:4298 (1974); Amzel, et al., *Proc Natl Acad Sci USA* 71:1427 (1974), de la Paz, et al., *EMBO J* 5:415 (1986); Kieber-Emmons, et al., *Int Rev Immunol* 2:339 (1987)) and then synthesizing conformationally restricted cyclic organic peptides which have the required contact residues and

conformation (Kahn, et al., *J Mol Recognition* 1:75 (1988); Kahn, et al., *J Am Chem Soc* 110:1638 (1988). Such mimetics can be prepared from the neutralizing antibodies produced by the mammalian hosts of the present invention and utilized in treatment of viral infections.

Furthermore, an isolated neutralizing antibody can be mass-produced by biomolecular sequencing techniques. The neutralizing antibody is first sequenced, and the sequence is then used as a template for in vitro production.

Example IV: Immunological Barrier and Preventatives

An immunological barrier to the transmission or invasion of SIV, HIV, or other similar viruses via the mucosal surfaces may be prepared using milk and/or serum neutralizing antibodies extracted as described in Example II. These antibodies are developed into a passive immunological barrier in the form of vaginal creams, rectal creams, eye drops, oral sprays, swabs or injections to be applied directly to the mucosa or at the site of accidental needle pricks or sticks. It should be noted that with oral, vaginal or rectal sex, a latex condom should be used concurrently to achieve maximum benefit but is not necessary if sufficient cream or spray is utilized.

The serum from the mammal is treated with ammonium sulfate to precipitate the neutralizing antibodies.

class and as well as complement fixing antibodies and neutralizing antibodies (hereinafter referred to as "serum S1"). Placement of the serum S1 into the body of the human serum recipient (H2) kills the active AIDS virus in the human serum recipient. Serum S1 also cross reacts with other HIV virus strains in the body of the human serum recipient (H2) in accord with standard virological reactions thereby yielding further serums upon proper treatment. This concept is expandable because the human donor's (H1) serum S1 now contains competent antibodies which cross react with his own parent or original HIV and similar sero-typed SIV and/or HIV viruses. The result is a serum which will block further development of the AIDS virus in both the human donor (H1) and human serum recipient (H2).

When the human donor's (H1) serum S1 is placed into an AIDS patient who has been sero-typed with the human donor, it allows the AIDS patient's body to respond to the passive transfusion of a competent antibody of serum S1 directed against the AIDS virus, thereby blocking the further development and replication of the AIDS virus. When the human serum recipient's (H2) immune system recovers to an extent as reflected in the "CD4" count from receiving serum S1, he will be eligible to receive the vaccine developed from the AAV2. The vaccine promotes the active production of competent antibodies in vivo in the human serum recipient which then yields serum or a competent

active immune response. It should be added that the antibodies isolated from the goat serum specifically for HIV now can be used intravenously for treatment of active AIDS complex disease.

It should be noted that an AAV2 vaccine can be produced from an HIV-positive human donor according to the procedures outlined in Example II, stored under appropriate under conditions of -20°C for long-term storage (≥30 days) or ≤40°C for short-term storage (<30 days), and then given to a human recipient suffering from an infection with the same sero-typed HIV virus at a later time. This would permit the preparation of an inventory of vaccines for various HIV sero-types to be subsequently given to patients upon diagnosis and sero-typing of the HIV virus.

Example VI: Agricultural Function

A cure or arresting mechanism to the caprine encephalitis (CAE) virus is now disclosed.

The principles described herein and the processes set out in Examples II and III are also equally applicable to the CAE virus which presently affects goats or other virulent viral systems in the same manner that the SIV and HIV viruses do humans. The mammal of choice for antibody production is the milk-producing cow. Use of these principles and techniques set forth above a will result in a program that should

Then, the antibodies will be dissolved in an isotonic solution. Once dissolved, the solution is maintained at ≤40°C until used. Long term storage, i.e., in excess of thirty days, can be achieved at temperatures of -20°C.

Thereafter, sprays, gels, creams, drops and similar applications can be manufactured using standard, acceptable industrial suspension and preservation technology.

Condom use can be either by manual application of the preparation to the interior of the condom prior to its use or by preapplication to the condom before packaging. Concurrent use of the preparation in the form of a vaginal cream or spray is recommended to enhance the barrier protection.

The average number of viral particles per milliliter of bodily secretions for which the barrier is intended can be determined by measuring the number for a particular patient, or for a population of patients. Once the average number is determined, then a theoretical neutralization number can be obtained using Table 2.

A human serum or a serum complex composed of neutralizing antibodies obtained from a mammal similar to, but not limited to, that described herein to arrest further cellular or viral development within the human body as set out may be developed for and against a specific SIV, HIV and/or other viruses, or mutated

strain(s) of these viruses. The serum development is applicable for humans and other species. The mammal's neutralizing antibodies which have been produced as described in Example II and which have been processed and stored in solution as described in Example III can be introduced into humans who have the HIV virus or other viruses under treatment. The serum is introduced preferably intravenously.

Example V: Preparation and Use of Serum or Serum Complex

A mammal's neutralizing antibodies, which have been produced, precipitated, and stored in solution as described in Examples II and III above, can be introduced into humans who have the HIV virus or other viruses under treatment. The serum is preferably introduced intravenously.

A human donor (H1) who has been diagnosed as being HIV-positive and whose HIV virus is sero-typed to match that of the expected human serum recipient (H2) is utilized. A vaccine AAV2 containing nonhuman mammal's neutralizing antibodies made from exposing the mammal to H1's HIV virus as described in Example II and subsequently processed and stored as given in Example III is introduced into the human donor (H1) preferably by intravenous injection. Upon receiving AAV2, the human donor's (H1) serum is then developed, as described in Example II and Example III, to produce a new serum having competent antibodies of the IgM-IgG

on proper application eradicate the CAE virus from the goat herds.

Example VII: Universal Application for All Viruses

The principles described herein and the processes set out are equally applicable to all viruses.

I claim:

1. A method for producing neutralizing antibodies for the treatment of a viral infection in a human patient, comprising the steps of:

a. exposing a mammal to a virus such that said mammal produces neutralizing antibodies to said virus and

b. collecting said neutralizing antibodies from said mammal.

2. The method of Claim 1, wherein said neutralizing antibodies comprises IgA.

3. The method of Claim 1, wherein said neutralizing antibodies comprises IgG1.

4. The method of Claim 1, wherein said virus is human immunodeficiency virus.

5. The method of Claim 2, wherein said virus is human immunodeficiency virus.

6. The method of Claim 3, wherein said virus is human immunodeficiency virus.

7. The method of Claim 1, wherein said virus is simian immunodeficiency virus.

8. The method of Claim 2, wherein said virus is simian immunodeficiency virus.

9. The method of Claim 3, wherein said virus is simian immunodeficiency virus.

10. A method for treating a viral infection in a human patient, comprising the steps of:

a. exposing a mammal to a virus such that said mammal produces neutralizing antibodies to said virus;

b. collecting said neutralizing antibodies from said mammal; and

c. introducing said neutralizing antibodies into said human patient.

11. The method according to Claim 10, wherein said neutralizing antibodies comprises IgA.

12. The method according to Claim 10, wherein said neutralizing antibodies comprises IgG1.

13. The method of Claim 10, wherein said virus is human immunodeficiency virus.

14. The method of Claim 11, wherein said virus is human immunodeficiency virus.

15. The method of Claim 12, wherein said virus is human immunodeficiency virus.

16. The method of Claim 10, wherein said virus is simian immunodeficiency virus.

17. The method of Claim 11, wherein said virus is simian immunodeficiency virus.

18. The method of Claim 12, wherein said virus is simian immunodeficiency virus.

19. A virus neutralizing agent for the treatment of a viral infection in a human patient, wherein said viral neutralizing agent is produced by exposing a mammal to said virus to produce said neutralizing agent to said virus, said viral neutralizing agent then collected from said mammal and introduced into said human patient.

20. The virus neutralizing agent of Claim 19, wherein said virus neutralizing agent comprises IgA antibodies isolated from serum collected from said mammal.

21. The virus neutralizing agent of Claim 19, wherein said virus neutralizing agent comprises IgG1 antibodies isolated from serum collected from said mammal.

22. The virus neutralizing agent of Claim 19, wherein said virus neutralizing agent comprises IgA antibodies isolated from milk collected from said mammal.

23. The virus neutralizing agent of Claim 19, wherein said virus neutralizing agent comprises IgG1 antibodies isolated from milk collected from said mammal.

24. A method for neutralizing a first virus expected to contact an area on a human by applying a virus neutralizing agent to said area, said virus neutralizing agent comprising neutralizing antibodies isolated from a mammal exposed to a second virus, said second virus being the same or similar to said first virus.

25. The method of Claim 24, wherein said virus neutralizing agent further comprises a pharmacologically acceptable carrier.

26. The method of Claim 24, wherein said first virus is human immunodeficiency virus and said second virus is human immunodeficiency virus of the same serotype as said first virus.

27. The method of Claim 25, wherein said first virus is human immunodeficiency virus and said second virus is human immunodeficiency virus of the same serotype as said first virus.

28. The method of Claim 24, wherein said neutralizing antibodies isolated from a mammal exposed to more than one virus.

29. The method of Claim 25, wherein said neutralizing antibodies isolated from a mammal exposed to more than one virus.

30. The method of Claim 24, wherein said area is mucosal surfaces.

31. The method of Claim 25, wherein said area is mucosal surfaces.

32. The method of Claim 26, wherein said area is mucosal surfaces.

33. The method of Claim 27, wherein said area is mucosal surfaces.

34. The method of Claim 28, wherein said area is mucosal surfaces.

35. The method of Claim 29, wherein said area is mucosal surfaces.

36. A method for neutralizing a first virus expected to contact an exterior or interior surface or both exterior and interior surfaces of a condom by applying a virus neutralizing agent to said exterior or interior surface or both exterior and interior surfaces of said condom, said virus neutralizing agent comprising neutralizing antibodies isolated from a mammal exposed to a second virus, said second virus being the same or similar to said first virus.

37. The method of Claim 36, wherein said virus neutralizing agent further comprises a pharmacologically acceptable carrier compatible with latex or rubber materials.

38. The method of Claim 36, wherein said first virus is human immunodeficiency virus and said second virus is human immunodeficiency virus of the same serotype as said first virus.

39. The method of Claim 37, wherein said first virus is human immunodeficiency virus and said second virus is human immunodeficiency virus of the same serotype as said first virus.

40. The method of Claim 36, wherein said neutralizing antibodies isolated from a mammal exposed to more than one virus.

41. The method of Claim 37, wherein said neutralizing antibodies isolated from a mammal exposed to more than one virus.

42. A method for neutralizing a first virus expected to contact an exterior or interior surface or both exterior and interior surfaces of a latex rubber glove by applying a virus neutralizing agent to said exterior or interior surface or both exterior and interior surfaces of said glove, said virus neutralizing agent comprising neutralizing antibodies isolated from a mammal exposed to a second virus, said second virus being the same or similar to said first virus.

43. The method of Claim 42, wherein said virus neutralizing agent further comprises a pharmacologically acceptable carrier compatible with latex or rubber materials.

44. The method of Claim 41, wherein said first virus is human immunodeficiency virus and said second virus is human immunodeficiency virus of the same serotype as said first virus.

45. The method of Claim 42, wherein said first virus is human immunodeficiency virus and said second virus is human immunodeficiency virus of the same serotype as said first virus.

46. The method of Claim 41, wherein said neutralizing antibodies isolated from a mammal exposed to more than one virus.

47. The method of Claim 42, wherein said neutralizing antibodies isolated from a mammal exposed to more than one virus.

48. A method for neutralizing caprine encephalitis virus in livestock, comprising contacting said caprine encephalitis virus with anti-caprine encephalitis virus neutralizing antibodies formed by an immunocompetent nonhuman mammal upon exposure to caprine encephalitis virus.

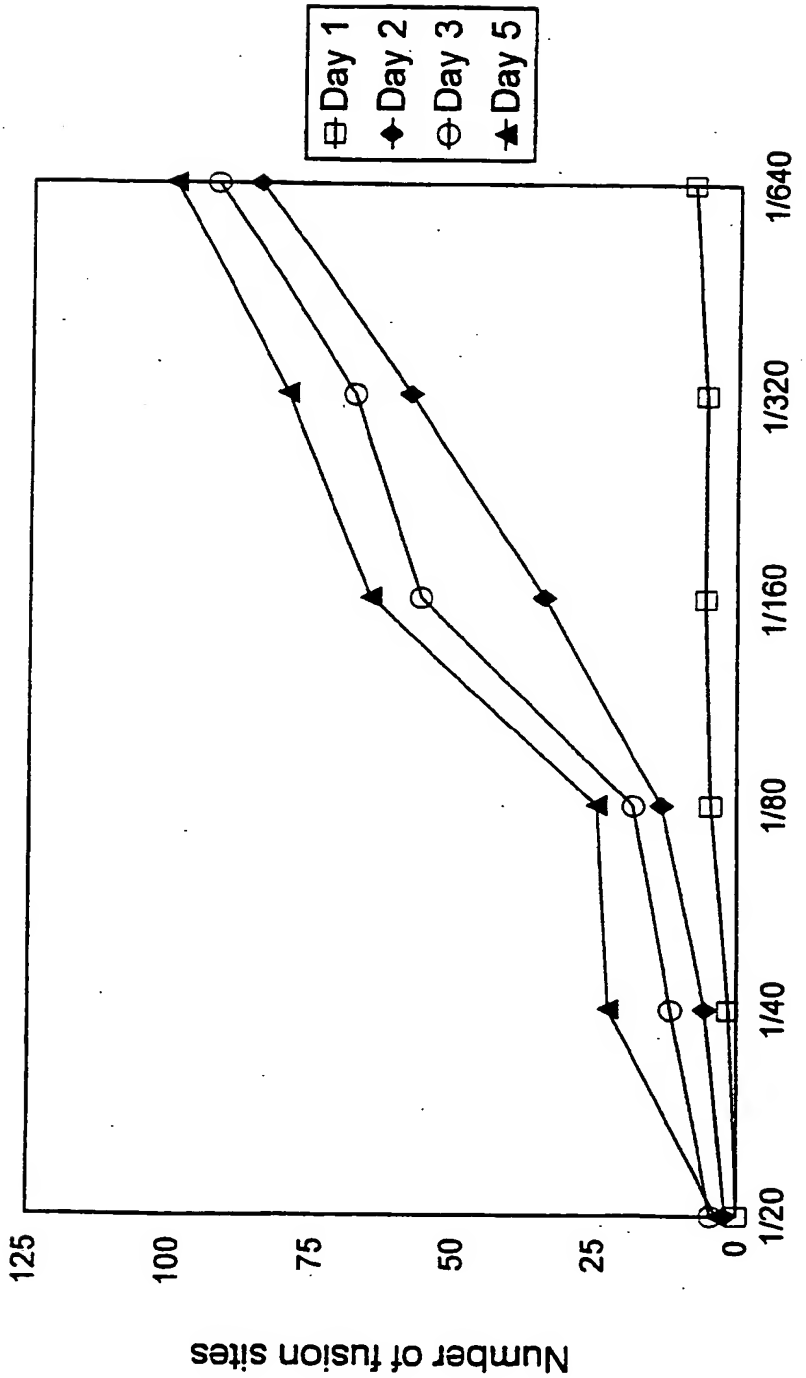
Day 1
Day 2
Day 3
Day 5
Number of fusion sites
125
100
75
50
25
0
1/20
1/40
1/80
1/160
1/320
1/640
Dilution of Goat anti-SIV serum

Fig. 1a

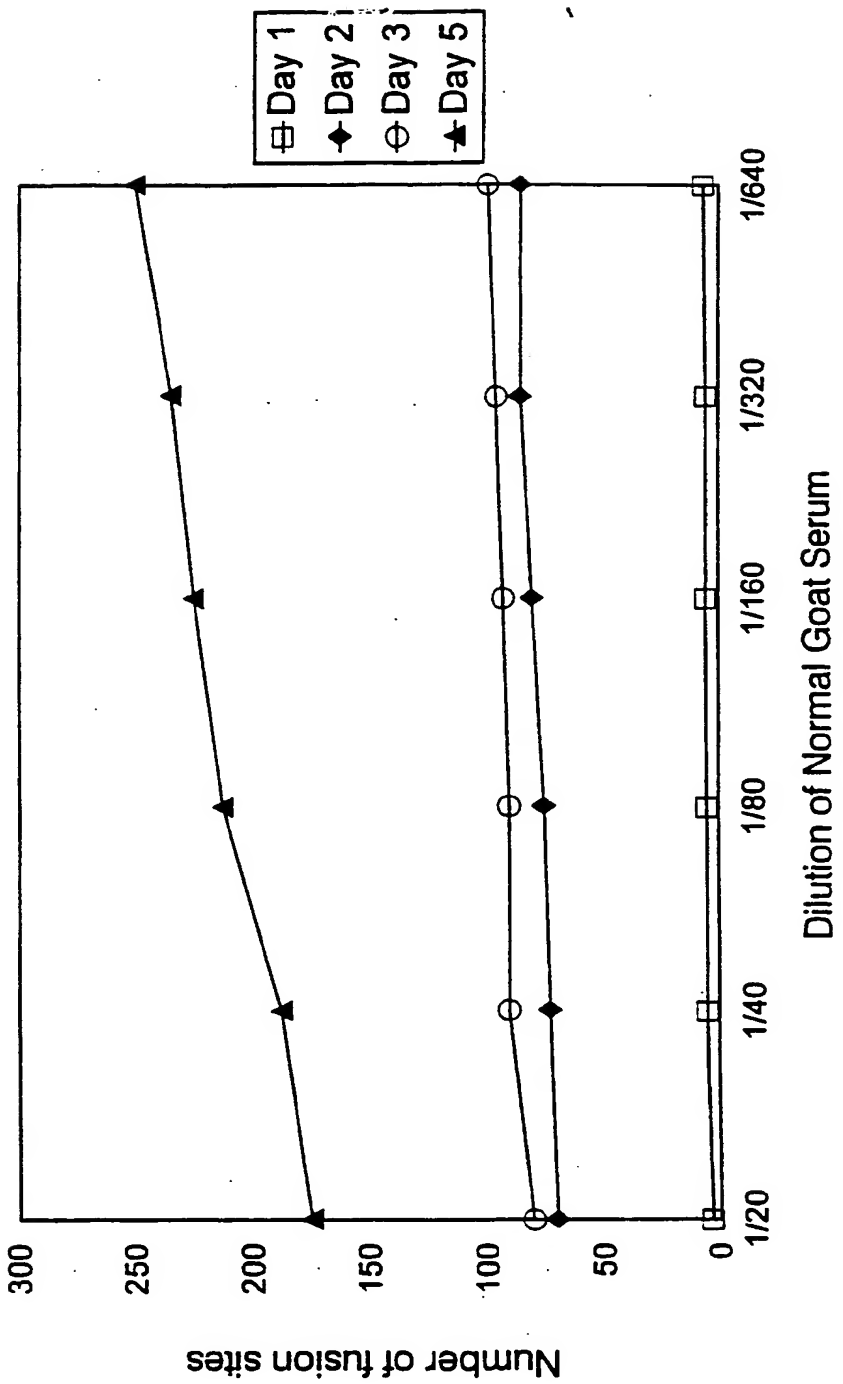
Day 1
Day 2
Day 3
Day 5
Number of fusion sites
300
250
200
150
100
50
0
1/20
1/40
1/80
1/160
1/320
1/640
Dilution of Normal Goat Serum

Fig. 1b

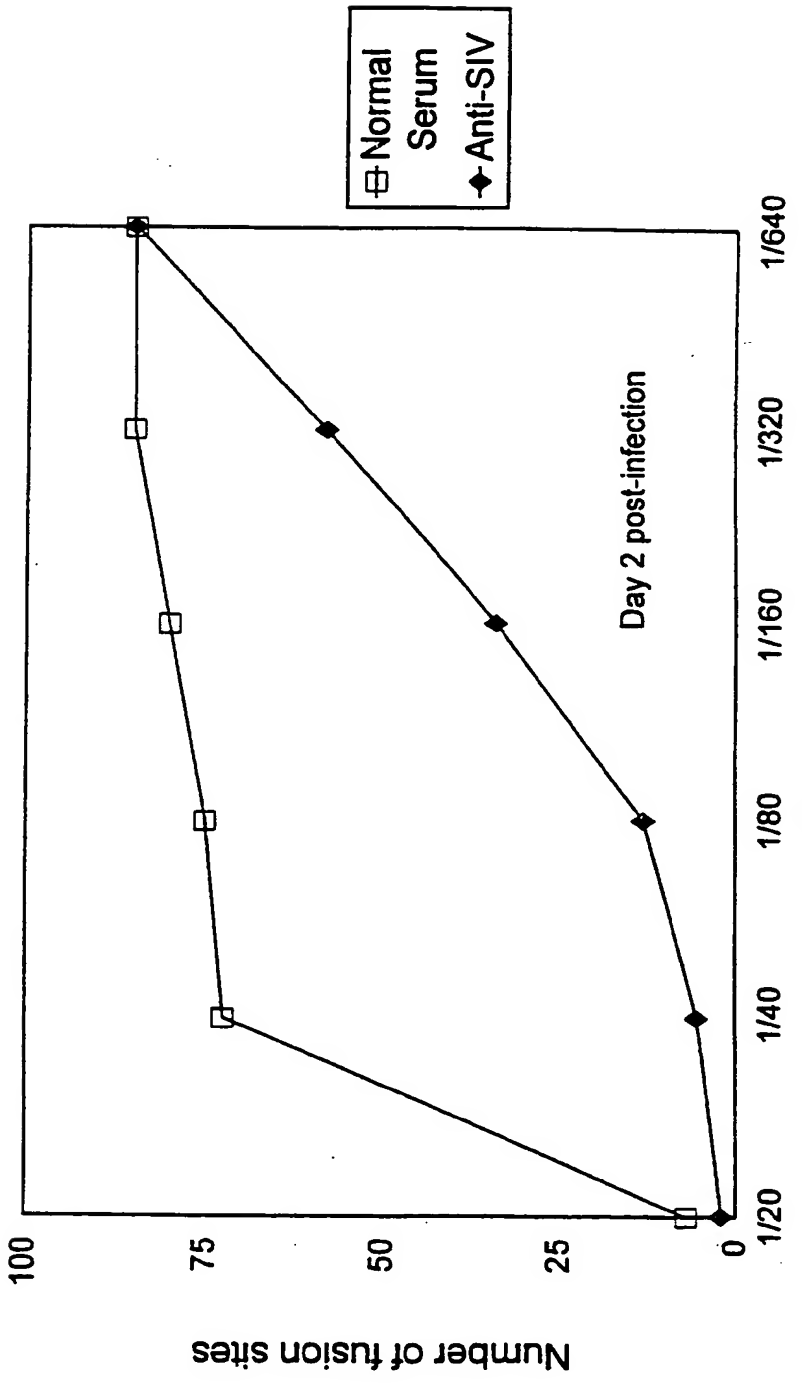
Normal Serum
Anti-SIV
Day 2 post-infection
Number of fusion sites
100
75
50
25
0
1/20
1/40
1/80
1/160
1/320
1/640
Serum Dilution

Fig. 2a

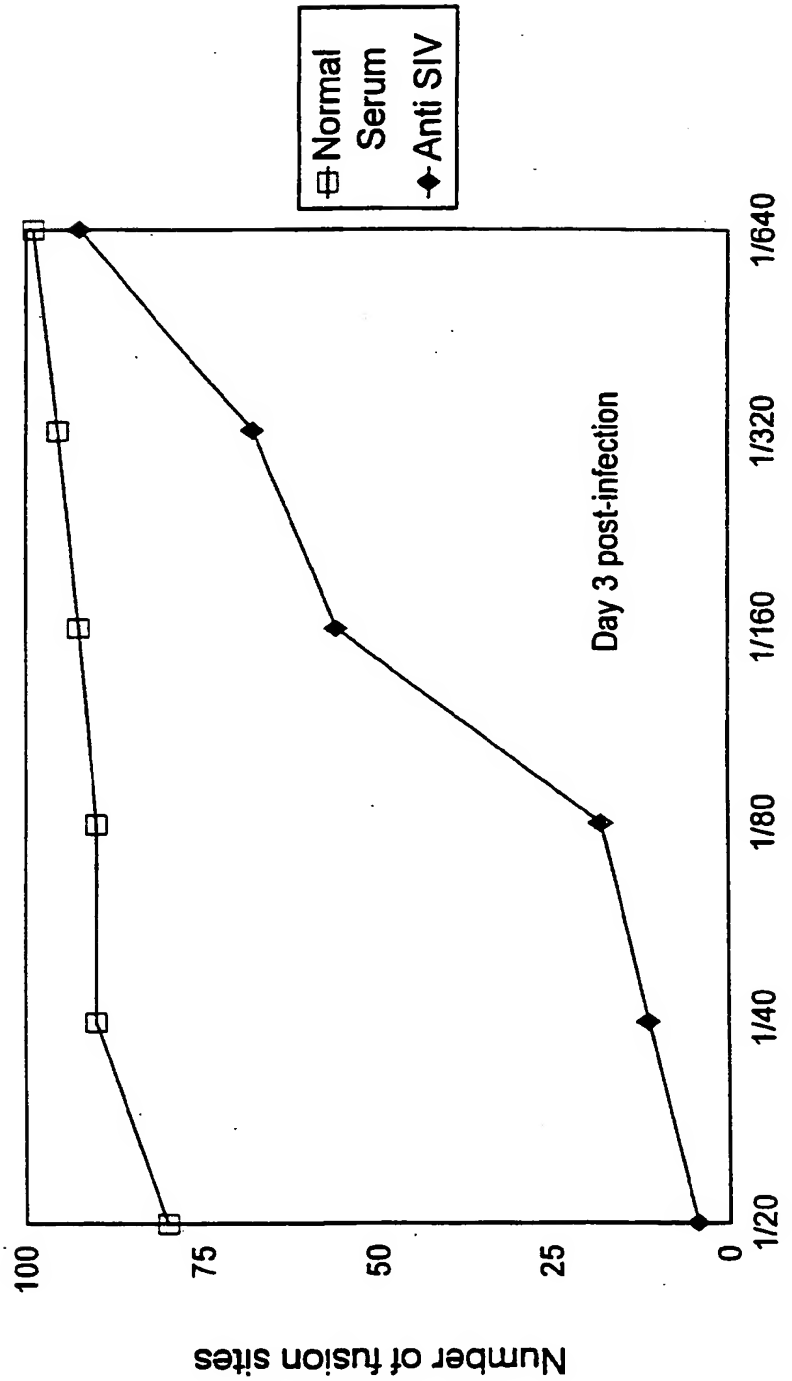
Normal Serum
Anti SIV
Day 3 post-infection
Number of fusion sites
0
25
50
75
100
1/20
1/40
1/80
1/160
1/320
1/640
Serum Dilution

Fig. 2b

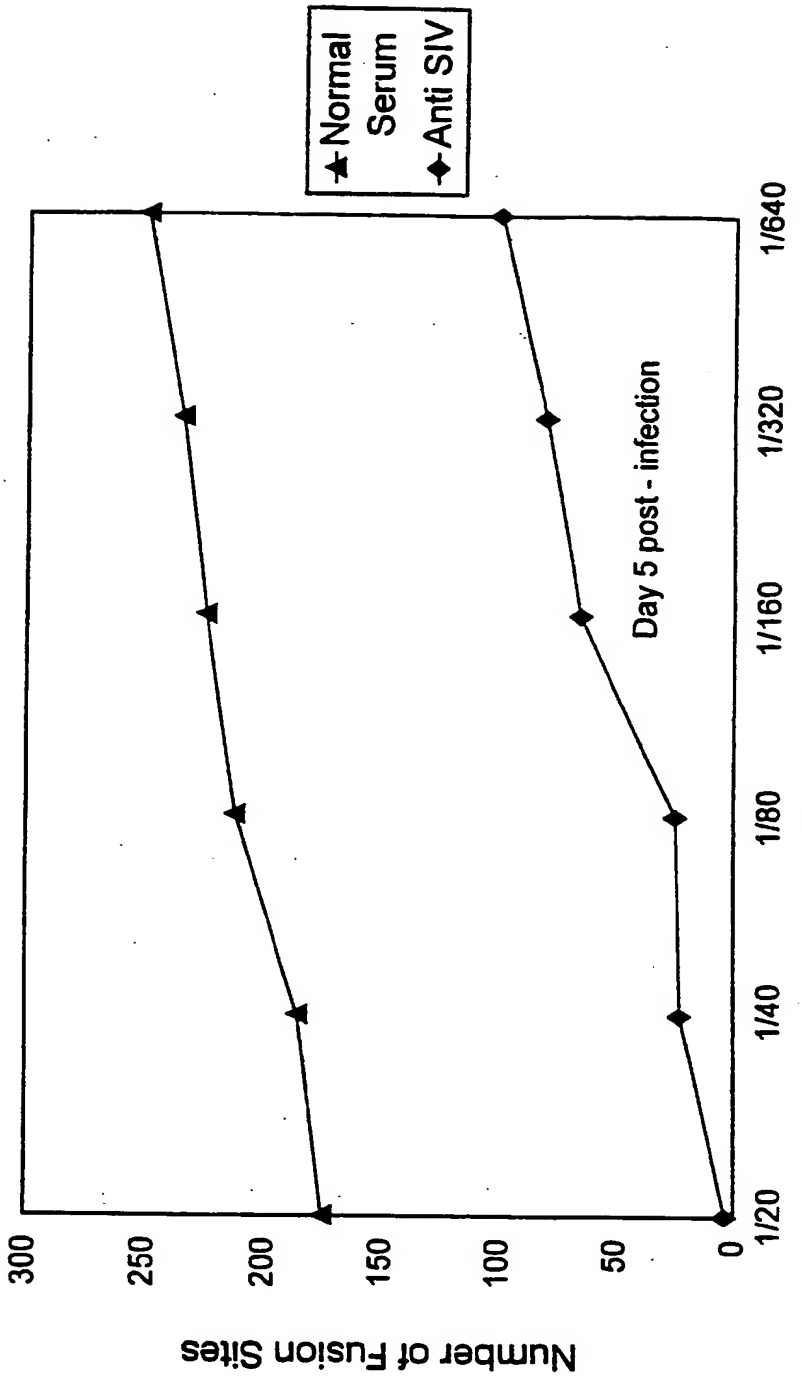
Normal Serum
Anti SIV
Day 5 post - infection
Number of Fusion Sites
300
250
200
150
100
50
0
1/20
1/40
1/80
1/160
1/320
1/640
Serum Dilution

Fig. 2c

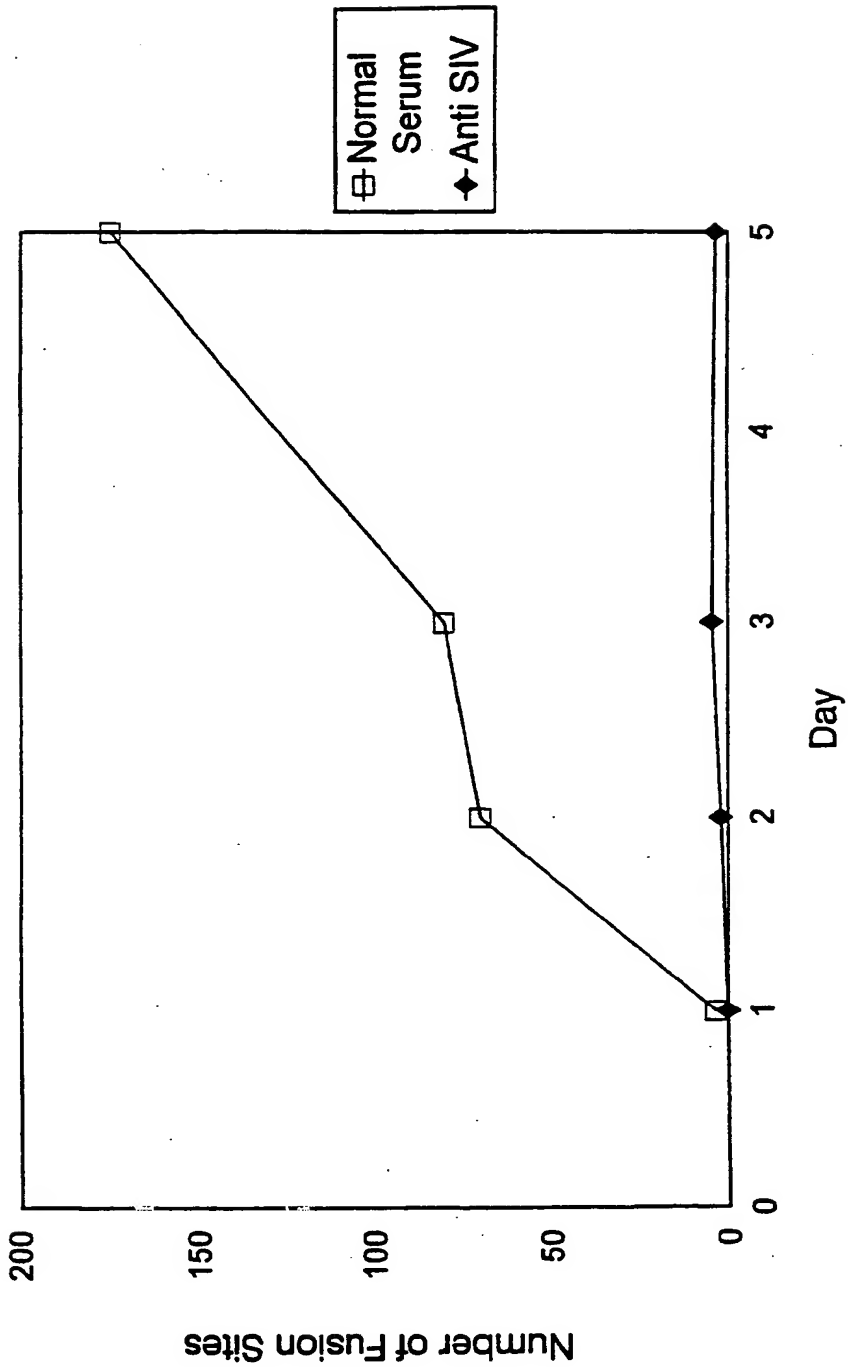
Normal Serum
Anti SIV
Number of Fusion Sites
200
150
100
50
0
0
1
2
3
4
5
Day

Fig. 3

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/11612

A. CLASSIFICATION OF SUBJECT MATTER
IPC(6) :A61K 39/42, 39/12, 45/00
US CL :424/159.1, 160.1, 204.1, 208.1, 278.1
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/159.1, 160.1, 204.1, 208.1, 278.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, DIALOG

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X -- Y	US, A, 5,143,727 (EBINA) 01 September 1992, column 1, Brief Description of the Drawing, figure 1, also Abstract.	1-3, 10-12, 19, 22 and 23 --- 1-6, 10-15, 19-23
X -- Y	Nature Medicine, Volume 1, No. 7, issued July 1995, H. Bukawa et al, "Neutralization of HIV-1 by secretory IgA induced by oral immunization with a new macromolecular multicomponent peptide vaccine candidate", pages 681-685, especially page 682, column 2, lines 3-6.	19-21 --- 1-6, 10-15, 19-23

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
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16 SEPTEMBER 1996	0 2 OCT 1996
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer HANKYEL T. PARK, PH.D. Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/11612

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Acta Paediatric JPN, Vol. 26, No. 1, issued 1984, S. Chiba et al, "Effect of exogeneous secretory IgA on chronic polivirus infection in a patient with agammaglobulinemia", pages 45-50, see abstract.	1-6, 10-15, 19-23
Y	US, A, 3,911,108 (SINGH, DECEASED) 07 October 1975, see Abstract	1-6, 10-15, 19-23
A	Clin. Exp. Immunol. Volume 88, issued 1992, J.L. Fahey et al., "Status of immune-based therapies in HIV infection and AIDS", pages 1-5.	1-6, 10-15, 24-27, 32-33
A	Bio/Technology, Volume 12, issued 12 February 1994, J.L. Fox, "No winners against AIDS", page 128.	1-6, 10-15, 24-27, 32-33
Y	Vaccine, Volume 9, issued November 1991, M. Gardner, "SIV vaccines: current status. The role of the SIV-macaque model in AIDS research", pages 787-791, see entire document.	24-35
Y	J. Med. Primatol. Vol. 21, issued 1992, M.L. Marthas et al, "Efficacy of live-attenuated and whole-inactivated simian immunodeficiency virus vaccines against vaginal challenge with virulent SIV", pages 99-107, see entire document.	24-35

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/11612

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☒ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
1-6, 10-15, 19-23, 24-35

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/11612

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I, claim(s) 1-23, drawn to a virus neutralizing agent (1st product), a method of producing neutralizing antibodies (1st method of making) and a method of treating a viral infection (1st method of using). Also contains the following species: (A) HIV, claims 4-6 and 13-15; (B) SIV, claims 7-9, 16-18.
Group II, claim(s) 24-35, drawn to a method for neutralizing a virus (2nd method of use).
Group III, claim(s) 36-47, drawn to a method for neutralizing a virus (3rd method of use).
Group IV, claim 48, drawn to a method for neutralizing caprine encephalitis virus in livestock (4th method of use).

The inventions listed as Groups I-IV do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: The methods of Groups I, II, III and IV each differ one from another in method steps , reagents, and utility and do not share a special technical feature. This application contains claims directed to more than one species of the generic invention. These species are deemed to lack Unity of Invention because they are not so linked as to form a single inventive concept under PCT Rule 13.1. The above mentioned species are directed to viruses which differ in their chemical, physical and immunologic properties as well as in their virulence and therefore do not share a special technical feature.

CORRECTED VERSION*

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
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With international search report.

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(54) Title: VIRAL SUPPRESSION, TREATMENT AND PREVENTION OF VIRAL INFECTIONS

(57) Abstract

It has been discovered that it is possible to administer neutralizing antibodies produced by a first mammal into a second mammal for the purpose of treating or preventing viral infections, with the first and second mammals being of either the same or different species. The neutralizing antibodies are mixed with the virus of interest such that the neutralizing antibodies bind at least one but not all epitopes of the virus so as to render the virus noninfectious while maintaining immunogenicity. The neutralizing antibodies may be administered as a serum transfusion, a vaccine, or a topical preparation.

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